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16. ABSTRACT

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Evidence that Microorganisms Cause Inactivation of Viruses in Activated Sludge

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Virus loss in activated sludge appeared to be caused by microorganisms. This conclusion is supported by the finding that poliovirus infectivity decreased during incubation in mixed-liquor suspended solids, primarily because of a sedimentable, heat-sensitive component. Furthermore, broth spiked with mixed-liquor suspended solids acquired antiviral activity during incubation.

Reuse of sewage and its components is becoming a popular method of resource conservation. Processes used to treat these waste products must insure that their reuse does not create an unacceptable risk to public health. One such process, activated-sludge treatment, has been shown to cause a significant reduction in sewage pathogens. For example, this process accompanied by a sedimentation step can remove more than 99% of the enteric viruses detectable in wastewater (24).

Initial studies on virus loss during activated sludge treatment suggested that association with wastewater particulates, which subsequently settle and become components of sludge, is the main mechanism of virus removal (6, 7, 12, 14–16). This would not be unexpected, because it has been clearly established that viruses added to wastewater rapidly become bound to particulate matter (11, 20, 23, 25, 26). The possibility that some fraction of virus loss during activated sludge treatment is due to inactivation was also suggested in these early reports. Further evidence that viruses are truly inactivated during this treatment process has been provided by more recent studies (10, 18).

Accelerated viral inactivation reportedly occurs in a number of other environmental systems. River water has been shown to have antiviral components (1, 21), and a number of reports describe rapid virus inactivation in marine waters (2, 3, 13, 17, 19). Several different causes of virus loss have been postulated from these studies. One proposed mechanism is inactivation by the direct or indirect action of microorganisms. Environmental isolates of bacteria with antiviral activity have been found on several occasions (8, 9; R. S. Fujioka, P. C. Loh, and L. S. Lau, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, N33, p. 178).

A possible mechanism of virus loss during activated sludge treatment is inactivation by microorganisms. In fact, several strains of bacteria with some antiviral activity were isolated in one of the earliest reports on virus removal during this process (12). Because optimization of natural disinfection methods is highly desirable, it is important to understand the role played by microorganisms in virus removal during activated sludge treatment. This study was designed for that purpose.

The principle method chosen for this laboratory study involved seeding mixed-liquor suspended solids (MLSS) of activated sludge with a standard enteric virus and observing the loss of viral PFU over time. The virus used was the LSc2ab strain of poliovirus 1, originally supplied by Lederle Research Laboratories, Pearl River, N.Y. This virus was propagated in BGM cells and analyzed for infectivity by the plaque assay in HeLa cells (25). Preparations of this virus that contained 9×10^9 PFU/ml were diluted 100-fold into MLSS obtained from the Sycamore Sewage Treatment Plant, Sycamore Township, Ohio, or into other media as specified. Samples (30 ml) were distributed into 125-ml Erlenmeyer flasks and incubated at 26 ± 1°C on a rotating platform (180 rpm). The dissolved oxygen content in the flasks was maintained above 5.5 mg/liter by this procedure. Fractions from each flask were periodically withdrawn and stored at −20°C. The thawed samples were then serially diluted in nutrient broth and analyzed for recoverable PFU.

The first experiment was done to determine the effect of MLSS on the recovery of poliovirus after different periods of incubation. This experiment was repeated three times with three separate MLSS samples and yielded essentially iden-

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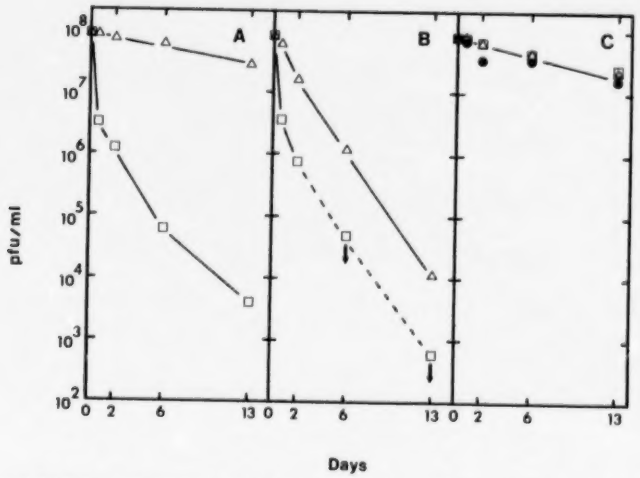

FIG. 1. Effects of MLSS, components of MLSS and disinfected components of MLSS on poliovirus recovery. (A) Poliovirus was diluted 100-fold into either MLSS (□) or phosphate-buffered saline (△), incubated at 26°C with aeration, and analyzed at the times specified for recoverable PFU. (B) Poliovirus added to the resuspended pellet (□) or to the supernatant fraction (△) of MLSS was incubated and analyzed for PFU. (C) Poliovirus added to the autoclaved pellet (●), autoclaved supernatant fraction (□), or filtered supernatant fraction (△) of MLSS was incubated and analyzed for PFU. Arrows indicate virus recoveries which were less than the specified values.

tical results. The data from one typical experiment (Fig. 1A) showed that a rapid decrease in recoverable PFU occurred during the first 16 h of incubation, relative to a control. The rate of loss during this period is comparable to those found by other investigators in model activated-sludge systems (4, 5, 10, 18, 22). The initial rate observed here decreased during extended aeration time, a result that would not have been evident in the model systems, in which retention times were relatively short.

The second experiment was done to determine the role of different MLSS components in virus loss. For this, the MLSS were centrifuged (27,000 × *g*, 20 min), the supernatant was removed, and the pellet was suspended in an equal volume of distilled water. This suspension and the supernatant fraction were then seeded with poliovirus. The loss of recoverable PFU was at least as rapid in the suspended pellet as in the original MLSS (Fig. 1B). On the other hand, the supernatant fraction initially (16-h sample) had almost no effect on virus recovery. However, expression of antiviral activity in this material became evident within 48 h and progressed steadily through the 13 days of incubation.

The third experiment was done to measure the effects of different disinfection methods on the antiviral activities in the pellet and supernatant fraction. A portion of the suspended pellet obtained in the previous experiment was autoclaved (121°C, 20 min), seeded with poliovirus, and examined for antiviral activity. At the same time, portions of the supernatant fraction were analyzed after being either autoclaved or filtered (0.22-μm filter). None of the three disinfected samples appeared to retain a significant amount of antiviral activity (Fig. 1C).

Taken together, these results indicated that MLSS contain a component(s) which inactivates poliovirus. This component appeared to be pelleted during centrifugation, destroyed by autoclaving, and removed by filtration. It seemed most likely that it was a microorganism or group of microbial species. However, it was also possible that the antiviral agent was a nonliving heat-sensitive material mainly associated with the particulate matter in activated sludge. The finding that the residual activity in the supernatant fraction after centrifugation appeared to increase over time tends to negate this possible explanation.

It was thought that if the antiviral component of activated sludge is a living organism, it may be possible to spike microbial growth medium with a small amount of MLSS to allow the production of this agent. To test this possibility, we seeded undiluted MLSS with poliovirus. At the same time, a sample of sterile broth (10 g of tryptone, 8 g of NaCl, 1 g of yeast extract, 1 g of dextrose, and 0.3 ml of 6 N NaOH per liter of distilled water), a sample of sterile broth spiked with MLSS (1 part MLSS per 99 parts broth), and a sample of broth spiked with the same

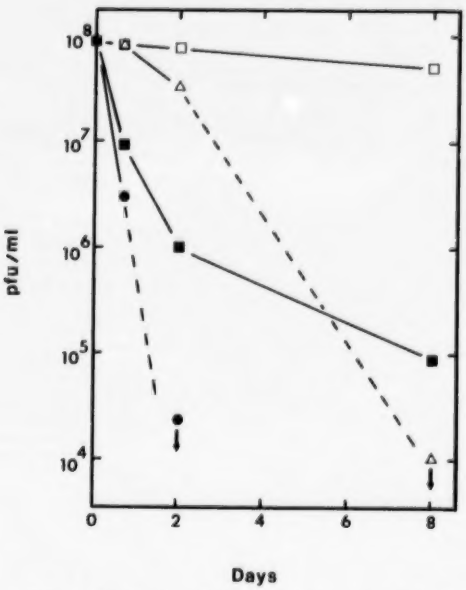

FIG. 2. Synthesis of the antiviral agent in broth spiked with MLSS. Poliovirus was diluted 100-fold into MLSS (■), broth (□), broth spiked with MLSS just before virus addition (△), or broth spiked with MLSS 3 days before virus addition (●) (dilution of MLSS into broth samples, 100-fold). After incubation with aeration at 26°C for the times specified, samples were withdrawn and analyzed for recoverable PFU. Arrows indicate virus recoveries which were less than the specified values.

amount of MLSS but incubated at 26°C with aeration for the previous 3 days were also seeded with poliovirus. The rates of loss of recoverable PFU were then compared in the four samples.

Poliovirus was very stable in the sterile broth, and the rate of virus loss in MLSS was comparable to that seen previously (Fig. 2). Broth freshly seeded with MLSS expressed no significant antiviral activity during the initial 16 h of incubation, but its viral titer was reduced to less than that found in MLSS after 8 days of incubation. Broth seeded with MLSS and incubated for 3 days before addition of virus caused a rapid loss of recoverable PFU within 16 h, an even greater loss than that observed in MLSS.

From these results it was concluded that the antiviral component of MLSS increases dramatically in broth. This finding, coupled with the previous findings that this component is pelleted during centrifugation, is sensitive to heat, and is removed by filtration, strongly indicates that it is a microorganism. The identity of this organism, the mechanism by which it decreases poliovirus recovery, and its effect on other enteric viruses remain to be determined.

LITERATURE CITED

1. **Akin, E. W., W. H. Benton, and W. F. Hill, Jr.** 1971. Enteric viruses in ground and surface waters: a review of their occurrence and survival. Proceedings of the 13th Water Quality Conference. Univ. Ill. Bull. **69**:59–74.
2. **Akin, E. W., W. F. Hill, Jr., and N. A. Clarke.** 1975. Mortality of enteric viruses in marine and other waters, p. 227–236. *In* A. L. H. Gameson (ed.), Discharge of sewage from sea outfalls. Pergamon Press, Ltd., Oxford, England.
3. **Akin, E. W., W. F. Hill, Jr., G. B. Cline, and W. H. Benton.** 1976. The loss of poliovirus 1 infectivity in marine waters. Water Res. **10**:59–63.
4. **Balluz, S. A., and M. Butler.** 1979. The influence of operating conditions of activated-sludge treatment on the behaviour of f2 coliphage. J. Hyg. **82**:285–291.
5. **Balluz, S. A., H. H. Jones, and M. Butler.** 1977. The persistence of poliovirus in activated sludge treatment. J. Hyg. **78**:165–173.
6. **Berg, G.** 1966. Virus transmission by the water vehicle. II. Virus removal by sewage treatment procedures. Health Lab. Sci. **3**:90–100.
7. **Clarke, N. A., R. E. Stevenson, S. L. Chang, and P. W. Kabler.** 1961. Removal of enteric viruses from sewage by activated sludge treatment. Am. J. Public Health **51**:1118–1129.
8. **Cliver, D. O., and J. E. Herrmann.** 1972. Proteolytic and microbial inactivation of enteroviruses. Water Res. **6**:797–805.
9. **Fujioka, R. S., P. C. Loh, and L. S. Lau.** 1980. Survival of human enteroviruses in the Hawaiian ocean environment: evidence for virus-inactivating microorganisms. Appl. Environ. Microbiol. **39**:1105–1110.
10. **Glass, J. S., and R. T. O'Brien.** 1980. Enterovirus and coliphage inactivation during activated sludge treatment. Water Res. **14**:877–882.
11. **Hurst, C. J., S. R. Farrah, C. P. Gerba, and J. L. Melnick.** 1978. Development of quantitative methods for the detection of enteroviruses in sewage sludges during activation and following land disposal. Appl. Environ. Microbiol. **36**:81–89.
12. **Kelly, S., W. W. Sanderson, and C. Neidl.** 1961. Removal of enteroviruses from sewage by activated sludge. J. Water Pollut. Control Fed. **33**:1056–1062.
13. **Lo, S., J. Gilbert, and F. Hetrick.** 1976. Stability of human enteroviruses in estuarine and marine waters. Appl. Environ. Microbiol. **32**:245–249.
14. **Lund, E., C. E. Hedstrom, and N. Jantzen.** 1969. Occurrence of enteric viruses in wastewater after activated sludge treatment. J. Water Pollut. Control Fed. **41**:169–174.
15. **Lund, E., and V. Ronne.** 1973. On the isolation of virus from sewage treatment plant sludges. Water Res. **7**:863–871.
16. **Mack, W. N., J. R. Frey, B. J. Riegle, and W. L. Mallmann.** 1962. Enterovirus removal by activated sludge treatment. J. Water Pollut. Control Fed. **34**:1133–1139.
17. **Magnusson, S., C. E. Hedstrom, and E. Lycke.** 1966. The virus inactivating capacity of sea water. Acta Pathol. Microbiol. Scand. **66**:551–559.
18. **Malina, J. F., Jr., K. R. Ranganathan, B. P. Sagik, and B. E. Moore.** 1975. Poliovirus inactivation by activated sludge. J. Water Pollut. Control Fed. **47**:2178–2183.
19. **Matossian, A. M., and G. A. Garabedian.** 1967. Virucidal action of sea water. Am. J. Epidemiol. **85**:1–8.
20. **Moore, B. E., B. P. Sagik, and J. F. Malina, Jr.** 1975. Viral association with suspended solids. Water Res. **9**:197–203.
21. **O'Brien, R. T., and J. S. Newman.** 1977. Inactivation of polioviruses and coxsackieviruses in surface water. Appl. Environ. Microbiol. **33**:334–340.

22. **Safferman, R. S., and M. E. Morris.** 1976. Assessment of virus removal by multi-stage activated sludge process. Water Res. **10**:413–420.
23. **Sattar, S. A., and J. C. N. Westwood.** 1976. Comparison of four eluents in the recovery of indigenous viruses from raw sludge. Can. J. Microbiol. **22**:1586–1589.
24. **Ward, R. L.** 1981. Virus removal during sludge treatment, p. 65–77. *In* M. Goddard and M. Butler (ed.), Viruses and wastewater treatment. Pergamon Press, Ltd., Oxford, England.
25. **Ward, R. L., and C. S. Ashley.** 1976. Inactivation of poliovirus in digested sludge. Appl. Environ. Microbiol. **31**:921–930.
26. **Ward, R. L., and C. S. Ashley.** 1979. Mode of initiation of cell infection with sludge-associated poliovirus. Appl. Environ. Microbiol. **38**:329–331.